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Dependence of spermatophore size and sperm number on body weight in various cricket species (Insecta, Orthoptera)

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A b s t r a c t : In several orthopteran species, spermatophore size and sperm number are known to correlate with male body size. As an ecologically important consequence of that, larger males are more successful than small males concerning both short-term, competitive mating and lifetime mating. In order to elucidate possible relationships between male body size and reproductive potential in field crickets, four species (*Teleogryllus commodus*, *Acheta domesticus*, *Gryllus bimaculatus*, *Gryllus assimilis*) were subjected to a morphometric and stereological investigation. For males of all four field crickets ampulla diameter significantly increases with body weight ($p < 0.001$), and sperm number positively correlates with the size of the spermatophore ($p < 0.05$). Highest numbers of spermatozoa per spermatophore are found in *G. assimilis*, whilst lowest numbers may be attested for *A. domesticus*. Interspecific comparisons confirm those results that were already obtained from intraspecific regression analysis. The results of the study suggest that large males are more successful in mating a female not only due to their more dominant behavior but also due to their ability to transfer larger sperm capsules with higher content of sperm cells. This clearly deviates from respective observations made in bush crickets, where reproductive costs are not invested into the amount of spermatozoa but into the size of the nuptial gift.

K e y w o r d s : Body weight, spermatophore, sperm number, ampulla, Orthoptera, crickets.

Introduction

In numerous insects, the sperm mass produced by the male is transferred to the female in a spermatophore during copulation. In general, this transport device may consist of two parts, the smaller sperm-containing ampulla as well as the larger jelly-like spermatophylax (Greek for 'sperm guard') that is devoid of sperm (DEWSBURY 1982, MANN 1984, REINHOLD & VON HELVERSEN 1997, STURM 2003). Regarding the spermatophores of male orthopterans and especially male crickets, production of a spermatophylax may not be observed among the entire range of species. Within the group of species providing a bipartite spermatophore (e.g. the bushcricket *Poecilimon veluchianus*), the spermatophylax commonly fulfills the function of a nuptial gift, distracting the female's attention from the sperm during the mating process (GWYNNE 1990, SIMONS 1990, SIMMONS & BAILEY 1990, HELLER & VON HELVERSEN 1991). Contrary to that, males of widely distributed field crickets (e.g. *Acheta domesticus* or *Gryllus bimaculatus*) do not deliver a spermatophylax as nuptial gift during the mating process; they instead apply alternative

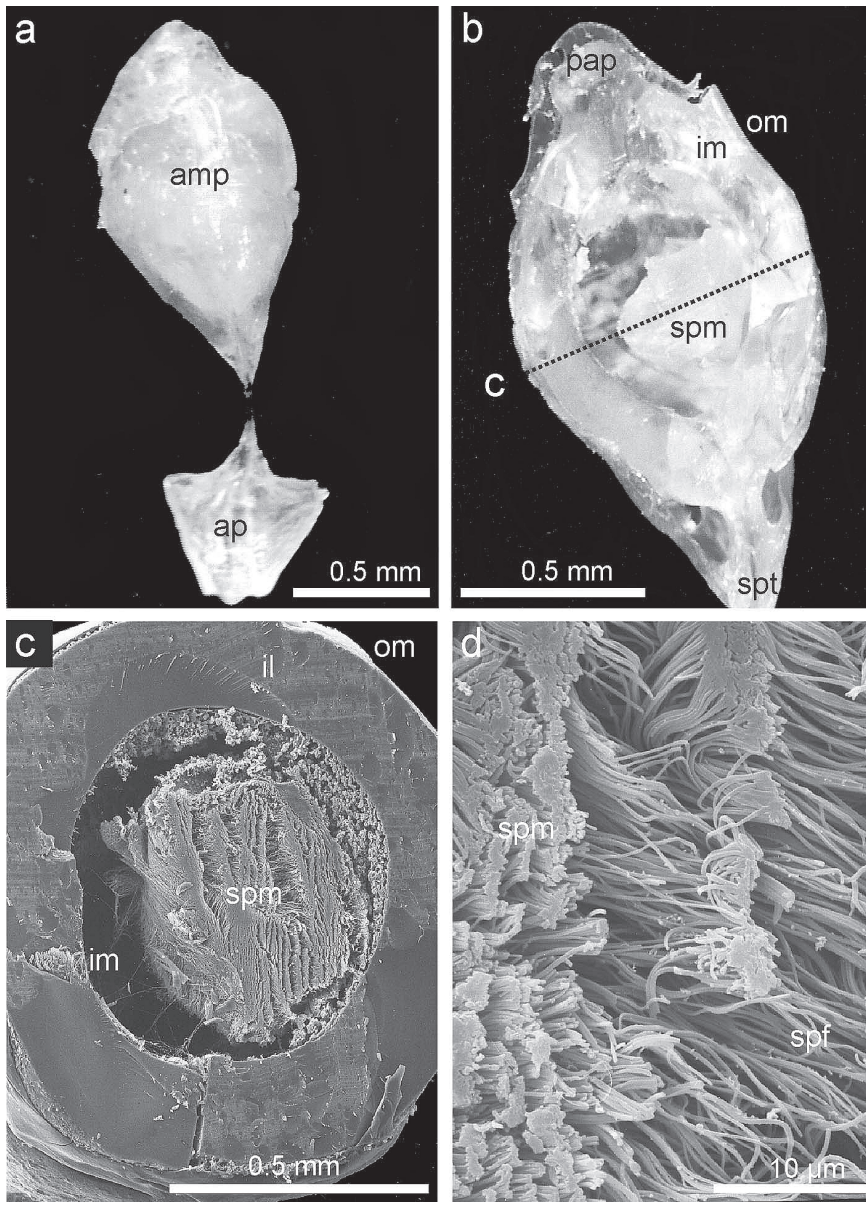

Fig. 1: General appearance of the spermatophore produced by males of the four cricket species investigated for this study (STURM 2003): (**a**) overview of a spermatophore with its sperm-containing ampulla (amp) and attachment plate (ap); (**b**) main components of the ampulla: apical papilla (pap), outer membrane (om), inner membrane (im), sperm mass (spm), and spermatphore tube (spt); (**c**) electron micrograph exhibiting the internal structure of a spermatophore (il: inner layer); (**d**) detailed view on the sperm mass included into the ampulla (spf: sperm flagella).

strategies such as guarding or food supply to distract the attention of their mating partners from the ampulla filled with sperm (STURM 2003; Fig. 1).

An interesting question concerns the amount of sperm being transferred into the female's receptaculum during a single mating process. According to previous studies, which have been carried out on various orthopterans, the number of germ cells per spermatophore significantly varies from species to species and thereby adopts values between several thousands and several millions (*Gryllodes supplicans*: 16,000-20,000, SAKALUK 1984; *Teleogryllus commodus*: 0.8-2 x 10^5, STURM 2003; *Kawanaphila nartee*: 0.2 x 10^6, SIMMONS & GWYNNE 1991; *Requiena verticalis*: 0.8-2 x 10^6, GWYNNE 1986; SIMMONS et al. 1993; *Poecilimon veluchianus*: 6.3-10.5 x 10^6, REINHOLD 1994; REINHOLD & VON HELVERSEN 1997). This high interspecific variability in sperm number has been assumed to correlate with the length of the time period between two spermatophore transfers or, in other words, the longer the intercopulatory interval, the higher the number of transferred spermatozoa (REINHOLD & HELLER 1993). Regarding the intraspecific variability in sperm number, besides the length of the intercopulatory interval also the physiological condition of the male, expressed by its body weight and body size, has been regarded as an essential controlling factor (STURM 2011).

In the study presented here, sperm number per delivered spermatophore is subjected to a detailed analysis for four cricket species (*T. commodus*, *A. domesticus*, *G. bimaculatus*, and *Gryllus assimilis*) which are characterized by very similar temporal distances between two copulations but partly remarkable differences with regards to their body dimensions. Possible relationships between spermatophore size (sperm number) and body size/weight are analyzed in order to evaluate the significance of this physiological factor for both intra- and interspecific variation in the amount of transferred germ cells.

Estimation of sperm numbers and their possible dependence on physiological factors represents a significant contribution to the reproductive biology of the studied cricket species. The information obtained from the analyses may help to increase our understanding of reproductive cycles in gryllids and the related role of intraspecific competition among males searching for a mating partner.

Materials and Methods

Crickets of the four species were reared under identical conditions (constant temperature of 25 °C, light : dark = 12 h : 12 h, relative humidity of 60 %) in a climate chamber at the former Institute of Zoology, University of Salzburg. Adult animals of each species were separated by gender and kept in 5-l glass vessels filled with crumpled paper. They were provided with food ad libitum, thereby offering standard diet for laboratory animals (Altromin© 1222), lettuce leaves as well as water that was contained in small dishes plugged with cotton wicks (STURM & POHLHAMMER 2000, STURM 2002).

Males of each species used for mating (N = 20) were subjected to a preceding measurement procedure, where body length (i.e. the total length of the three body segments without antennae and cerci) was determined by using a high precision (0.01 mm) slide caliper, whilst body weight was analyzed with a Satorius© balance (precision: 0.0001 g).

For the mating process, males were placed together with virgin females of the same species in respective mating vessels (round glass dishes with a diameter of 30 cm). Im-

mediately after copulation and spermatophore transfer, the sperm-containing capsules were removed from the females by using soft forceps. Separated spermatophores were submerged in insect Ringer's solution (STURM & POHLHAMMER 2000), liberated from any opaque mass surrounding the ampulla with modified, pincer-like forceps, and finally measured in detail under the stereomicroscope (Wild©).

For the estimation of sperm numbers contained in the spermatophores, isolated sperm capsules were fixed in a paraformaldehyde-glutaraldehyde mixture (KARNOVSKY 1965) for 3 h, washed in sodium-cacodylate buffer, dehydrated in a graded series of ethanol, and critical-point dried. After the fixation procedure, sperm capsules were subjected to an exact median section which was conducted with a razor blade under the microscope. The ventral halves were prepared for electron-microscopy (charging with carbon, sputtering with gold) and subsequently scanned with a Cambridge© 250 SEM at an accelerating voltage of 10-30 kV. Produced photographs were subject to a stereographic analysis insofar as they were covered with a 50x50-mesh grid, and the numbers of single sperm cells within single grid units were carefully counted. Total number of cells was computed by multiplying the amount of sperm per grid unit with the number of grid units covering the whole sperm mass (STURM 2011).

Physiological parameters (body size and body weight), dimensions of the isolated spermatophores, and sperm numbers contained in the separated capsules were analyzed statistically, thereby calculating most significant parameters of position and dispersion. In order to elucidate possible differences of mean values between the studied species, a parametric test (nested ANOVA, test for homogeneity according to Levene) followed by a post-hoc Bonferroni test was applied. Means were evaluated to differ significantly for $p < 0.05$ and to differ with high significance for $p < 0.001$. For the determination of possible relationships between physiological parameters and spermatophore size or sperm number, respective scatter plots were drawn and least-squares regression analyses were carried out. Constants and intersects of the regression lines indicating probable correlations between independent and dependent variable were tested for significance by using a suitable Student's t-test.

Results

Weight and length measurements conducted on the four cricket species clearly demonstrate that *A. domesticus* represents the smallest species of the study (weight: 723.8 $\pm$ 97.4 mg, length: 19.7 $\pm$ 1.9 mm, N = 20), whereas *G. assimilis* brings out the largest individuals (weight: 935.3 $\pm$ 165.7 mg, length: 24.1 $\pm$ 3.6 mm, N = 20; Tab. 1). The remaining two cricket species are characterized by body parameters plotting between those of *A. domesticus* and *G. assimilis*, whereby *G. bimaculatus* (weight: 865.7 $\pm$ 114.9 mg, length: 22.5 $\pm$ 2.4, N = 20) has to be regarded as sgnificantly larger than *T. commodus* (weight: 756.5 $\pm$ 102.6, length: 21.3 $\pm$ 2.2 mm, N = 20). Application of parametric test statistics resulted in significant differences ($p < 0.05$) of the measured body parameters between all species with three exceptions (Tab. 3): *A. domesticus* does not differ significantly in weight from *T. commodus*, *G. bimaculatus* does not differ significantly in weight from *G. assimilis*, and *T. commodus* does not differ significantly in length from *G. bimaculatus*.

Spermatophores of those males, which had been subjected to the measurements noted above, were explored for their size (measurement of the inner diameter of the ampulla)

and their content of sperm (Tab. 2). Regarding the diameter of the ampulla, representing the sperm-containing capsule of the spermatophore, a trend similar to that derived from body parameter measurements is obtained. Therefore, males of *A. domesticus* produce the smallest sperm capsule ($D_{ampulla}$ = 0.71 $\pm$ 0.07 mm, N = 20), whilst the respective sperm-transferring device produced by *G. assimilis* is significantly larger ($p < 0.05$; $D_{ampulla}$ = 0.89 $\pm$ 0.11). Males of *T. commodus* transfer a spermatophore to their mating partners whose ampulla is characterized by a diameter of 0.76 $\pm$ 0.09 mm, and males of the Mediterranean field cricket *G. bimaculatus* transfer a respective capsule with $D_{ampulla}$ = 0.83 $\pm$ 0.12 mm. Whilst values for $D_{ampulla}$ of *A. domesticus* and *T. commodus*, *T. commodus* and *G. bimaculatus* as well as *G. bimaculatus* and *G. assimilis* are characterized by insignificant discrepancies (Tab. 3), all other values listed in the respective cross table indicate noticeable differences. The number of sperm contained in the spermatophores commonly ranges from 1.23 x 10^5 $\pm$ 0.65 x 10^5 (*A. domesticus*) to 2.21 x 10^5 $\pm$ 1.05 x 10^5 (*G. assimilis*; Tab. 2). As exhibited in Tab. 3, significant differences of the number of transferred spermatozoa may be only attested between *A. domesticus* and *G. assimilis*.

Table 1: Physiological parameters measured for males of the four cricket species of this study ($N_{species}$ = 20). Abbreviations: SD = standard deviation, MIN = minimum, MAX = maximum.

Parameter	*T. commodus*	*A. domesticus*	*G. bimaculatus*	*G. assimilis*
Weight (mg)	756.5	723.8	865.7	935.3
SD	102.6	97.4	114.9	165.7
MIN	567.2	507.8	698.2	743.4
MAX	934.8	896.2	1056.5	1145.6
Length (mm)	21.3	19.7	22.5	24.6
SD	2.2	1.9	2.4	3.1
MIN	18.7	17.4	19.8	20.5
MAX	24.3	22.8	25.6	28.5

For a determination of possible intraspecific relationships between cricket body dimensions, expressed by the body weight, and spermatophore size, expressed by the inner diameter of the ampulla, respective linear regression analyses were conducted (Tab. 4). As underlined by the computed regression prameters, $D_{ampulla}$ (dependent variable) and body weight (independent variable) are marked by highly significant ($p < 0.001$) correlations in all cricket species examined for this study. Since general regression based upon the common equation $y = b_0 + b_1x$ resulted in positive values of y for x = 0, b_0 was set to 0 in this specific case. According to the results of regression analysis, $D_{ampulla}$ linearly increases with the body weight of the male crickets. In order to find out, whether the sperm number in a spermatophore correlates with spermatophore size and, as a consequence, with body weight of the crickets, another set of linear regression analyses was performed (Tab. 4). Again, a highly significant correlation ($p < 0.001$) between sperm number and $D_{ampulla}$ could be determined for all species studied. In contrast to the first regression set, the regression line was not assumed to cross the origin, resulting in a minimal $D_{ampulla}$, below which theoretically no filling of the spermatophore with sperm cells takes place anymore.

Table 2: Spermatophore dimensions and sperm numbers determined for the four cricket species (Nspecies = 20). Abbreviations: $D_{ampulla}$ = mean diameter of the ampulla, SD = standard deviation, MIN = minimum, MAX = maximum, Sperm = number of spermatozoa.

Parameter	*T. commodus*	*A. domesticus*	*G. bimaculatus*	*G. assimilis*
$D_{ampulla}$ (mm)	0.76	0.71	0.83	0.89
SD	0.09	0.07	0.12	0.11
MIN	0.65	0.61	0.69	0.75
MAX	0.88	0.83	0.96	1.03
Sperm (x 10^5)	1.56	1.23	1.76	2.21
SD	0.78	0.65	0.87	1.05
MIN	0.67	0.24	0.79	0.98
MAX	2.34	1.97	2.67	3.27

In order to obtain more generalized information of the relationship between sperm number and male body weight, a linear regression analysis including all four cricket species was carried out (Fig. 2). Concerning the correlation between $D_{ampulla}$ and body weight (Fig. 2a), $D_{ampulla}$ increases by 0.001 mm per each additional mg body weight ($p < 0.001$). The relationship between sperm number and $D_{ampulla}$ is described by a regression line with the intercept being located at -3.409 x 10^5 (standard error: 0.296 x 10^5) and the constant b_1 adopting a value of 6.449 x 10^5 (standard error: 0.369 x 10^5). Both regression coefficients are characterized by high significance ($p < 0.001$; Fig. 2b).

Table 3: Cross table for the presentation of one-way ANOVA results (df = 79, $p < 0.001$) of possible significant differences of parameter mean values among the studies species (* = significant with $p < 0.05$, ** = highly significant with $p < 0.001$).

Weight	*T. commodus*	*A. domesticus*	*G. bimaculatus*	*G. assimilis*
T. commodus	------			
A. domesticus	0.453	------		
G. bimaculatus	0.033*	0.000**	------	
G. assimilis	0.000**	0.000**	0.101	------
Length	*T. commodus*	*A. domesticus*	*G. bimaculatus*	*G. assimilis*
T. commodus	------			
A. domesticus	0.019*	------		
G. bimaculatus	0.447	0.000**	------	
G. assimilis	0.000**	0.000**	0.041*	------
$D_{ampulla}$	*T. commodus*	*A. domesticus*	*G. bimaculatus*	*G. assimilis*
T. commodus	------			
A. domesticus	1.000	------		
G. bimaculatus	0.134	0.002*	------	
G. assimilis	0.000**	0.000**	0.164	------

Sperm	*T. commodus*	*A. domesticus*	*G. bimaculatus*	*G. assimilis*
T. commodus	------			
A. domesticus	1.000	------		
G. bimaculatus	1.000	0.746	------	
G. assimilis	0.138	0.005*	0.359	------

Discussion

In the contribution presented here, males of four cricket species were analyzed according to three main aspects: First, it should be demonstrated, in what extent body parameters such as body length and body weight vary among the species; second, possible inter-specific discrepancies in spermatophore size and sperm number per spermatophore should be elucidated with the help of appropriate statistical methods; third, possible intra- and interspecific correlations between body parameters (weight) and spermatophore parameters (ampulla diameter, sperm number) should be found.

Table 4: Regression coefficients describing possible correlations between ampulla diameter and cricket weight as well as sperm number and ampulla diameter.

$D_{ampulla}$-weight	Coefficient	Std. Error	Std. Beta	t	p
T. commodus	0.001	0.000	0.999	101.5	0.000
A. domesticus	0.001	0.000	0.998	63.9	0.000
G. bimaculatus	0.001	0.000	0.998	66.2	0.000
G. assimilis	0.001	0.000	0.999	85.2	0.000
Sperm-$D_{ampulla}$	Coefficient	Std. Error	Std. Beta	t	p
T. commodus					
intercept	-378137.86	40923.79		-9.24	0.000
constant	717290.03	54265.56	0.952	13.22	0.000
A. domesticus					
intercept	-502896.24	83845.34		-5.99	0.000
constant	896941.48	118543.03	0.872	7.57	0.000
G. bimaculatus					
intercept	-373235.38	69007.13		-5.41	0.000
constant	661995.19	83457.03	0.882	7.93	0.000
G. assimilis					
intercept	-540235.38	69366.00		-7.79	0.000
constant	853032.06	77506.78	0.933	11.01	0.000

As documented by linear regression analysis, each cricket species exhibits a positive correlation between ampulla diameter and body weight as well as between sperm number and ampulla diameter. This leads to the conclusion that larger (heavier) males produce larger spermatophores containing higher numbers of spermatozoa. As already outlined by

STURM (2011), in males of *T. commodus*, the number of sperm enclosed in a spermatophore is positively correlated with body size. This result seems to be plausible insofar as body size may positively correlate with the dimensions of internal organs, rather than reflecting only variations in the fatty tissue content. As could be demonstrated for several bush cricket species, spermatophore weight and number of contained sperm as well as ampulla size and number of spermatozoa are subject to a positive correlation (WEDELL 1993, MCCARTNEY et al. 2008). However, due to high natural fluctuations in sperm number, MCCARTNEY et al. (2008) also suggest to be cautious when using ampulla weight (size) to predict the amount of ejaculated or transferred spermatozoa. Contrary to field crickets, bush crickets additionally produce a spermatophylax (see above), whose size exhibits a well definable inter-relationship with ampulla weight. It is commonly hypothesized that bush cricket species preferably invest their energy in the production of large nuptial gifts which help to protect the germ cells from any processes other than reproduction (ejaculate protection hypothesis).

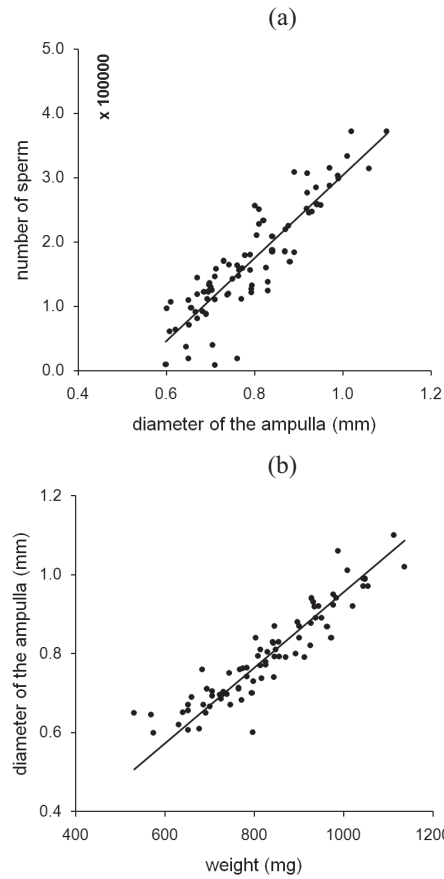

Fig. 2: Linear regression analyses elucidating possible relationships between ampulla diameter and body weight (**a**) as well as between number of sperm and ampulla diameter (**b**). Data obtained for all investigated cricket species have been plotted (N = 80).

From an ecological point of view, larger (heavier) males are regarded to possess a competitive advantage with respect to smaller males when fighting for access to females (THORNHILL & ALCOCK 1983). In order to balance their physiological deficit, small males frequently use specific tactics for gaining access to mating opportunities (JUDSON 2002). Such tactics may range from specific strategies concerning the fighting of mating competitors to the occupation of ecological niches that are commonly avoided by a respective species. In ecological respects, it has to be also mentioned that male crickets seem to have the ability to modify sperm number in response to both intraspecific competition and female size (GAGE & BARNARD 1996). In the present study, both factors were controlled as much as possible during the experiments reported on here (mating between one male and one female, selection of equally sized females). However, it has to be kept in mind that the same male is able to fill a spermatophore with the highest number of sperm possible or leave it completely empty. Therefore, ecological factors controlling the state of spermatophore filling have to be elucidated more in detail in future.

In the field cricket, *G. bimaculatus*, male size has been determined to remarkably influence short-term, competitive mating success (SIMMONS 1986, 1988). These short-term measures of mating success do not provide any information on the lifetime mating success and its possible dependence on male size. Laboratory experiments, however, could demonstrate that smaller males have to invest higher physiological costs into the production of spermatophores, resulting in a prolongation of the refractory period between mating and, consequently, the limitation of the reproductive potential. As another important factor, smaller males are mostly excluded from female choice of the mating partner and partly present as incompetent in terms of their ability to attach spermatophores once mounted (SIMMONS 1988). Comparable studies for other field crickets are still missing, although it has to be assumed that their behavior is highly similar to that of *G. bimaculatus*.

Although numerous studies support a positive correlation between male's size and short-term mating potential among several Orthopteran species, the question should be asked, whether females act in a similar way regarding their mating success. According to ecological and behavioral investigations published hitherto, the number of inseminated eggs released by female field crickets (especially in the case of *T. commodus* and *G. bimaculatus*) seems to be controlled by external rather than by physiological factors (HONĚK 1993, BRETMAN et al. 2006, JENNIONS et al. 2007). Whilst domination of a female by a male, with large males commonly being more dominant than small ones, positively correlates with the female egg-laying rate, polyandry combined with multiple mating of female only results in an insignificant increase of the reproductive potential.

Those phenomena being noticeable for the Orthoptera may be also documented for other insects, whereby most comprehensive observations have been documented for the Hymenoptera and the Lepidoptera. Regarding the first insect group, sperm numbers in drone honeybees clearly depend on body size (SCHLÜNS et al. 2003). Here, the higher investment in rearing of large males is outweighed by an increased mating potential, resulting in a larger progeny per male. In butterflies, male body size is verifiably interrelated with testis size and sperm length (GAGE 1994), underlining the enhanced mating potential of large males compared to small ones. In contrast to the Orthoptera treated above, female Lepidoptera also exhibit an influence of body size on egg dimensions and fecundity (BAUERNFEIND & FISCHER 2008). The latter finding makes sense insofar as

female insects flying mostly in the air may not be dominated by males in a similar way as female insects living on the ground.

Zusammenfassung

Von einigen Orthopterenarten ist im Allgemeinen bekannt, dass Spermatophorengröße und Anzahl der Spermien mit der Körpergröße der Männchen korrelieren. Als ökologisch bedeutsame Konsequenz dieser Tatsache ist größeren Männchen in Bezug auf das kurzzeitige kompetitive und lebenslange Paarungsverhalten mehr Erfolg beschieden als kleineren Männchen. Um mögliche Zusammenhänge zwischen männlicher Körpergröße und dem reproduktiven Potential in Feldgrillen herauszufinden, wurden vier Grillenarten (*Teleogryllus commodus*, *Acheta domesticus*, *Gryllus bimaculatus*, *Gryllus assimilis*) umfangreichen morphometrischen und stereologischen Untersuchungen unterzogen. Bei den Männchen aller vier Grillenarten nimmt der Ampulladurchmesser signifikant mit dem Körpergewicht zu ($p < 0.001$), und die Spermienzahl zeigt eine positive Korrelation mit der Größe der Spermatophore ($p < 0.05$). Die meisten Spermatozoen pro Spermatophore ließen sich bei *G. assimilis* nachweisen, während *A. domesticus* über die geringste Anzahl an Keimzellen pro Spermatophore verfügte. Interspezifische Vergleiche unterstreichen weitgehend jene Resultate, die bereits aus intraspezifischen Regressionsanalysen gewonnen werden konnten. Die Ergebnisse dieser Studie bestätigen einmal mehr, dass größere Männchen höheren Paarungserfolg bei Weibchen verbuchen, und dies nicht nur aufgrund ihrer dominanteren Verhaltensweisen, sondern auch infolge ihrer Fähigkeit zur Übertragung größerer Spermatophoren mit mehr Inhalt an Keimzellen. Dieser Sachverhalt weicht von entsprechenden Beobachtungen bei Buschgrillen ab, wo reproduktive Kosten nicht so sehr in die Menge an Spermatozoen, sondern viel mehr in die Größe des "Paarungsgeschenkes" investiert werden.

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